

ATTACHMENT B - CLEAN COPY OF SPECIFICATION

Please amend the specification at page 19, line 21:

N/E
Flagella are a key virulence determinant of Campylobacter spp. since motility is essential for establishment of colonization in the mucus lining of the gastrointestinal tract (25,26,27)

Please amend the specification at page 8, lines 13-21 through page 9, line 4:

B⁴
Region I of the flaA gene represents the highly conserved N terminal region, and regions II and III represent two regions which are more variable among different sequenced flagellin genes. Regions II and III are not, however, as variable as region IV. The construct was made by amplifying the regions I, II and III using the primer flaA-11 (5'ACCAATATTAACACAAATGTTGCAGCA3') (Seq. ID no. 3) and flaA-2 (5'TTATCTAGACTAATCTCTACCATCATTTTTAAC3') (Seq. ID no.4). The PCR product is digested with the appropriate restriction enzymes in order to insert the product into an expression vector. Any plasmid expression vector, e.g. PETTM(Novogen, Madison Wisconsin) or PMALTM (New England Biolabs, Beverly, MA) and viral expression vectors (e.g. adenovirus, M13, herpesvirus, vaccinia, baculovirus, etc) expression systems can be used as long as the polypeptide is able to be expressed. The PETTM vector is used for the cloning and over-expression of recombinant proteins in E.coli. In the PETTM system, the cloned gene is expressed under the control of a phage T7

promotor. In the PMALTM protein fusion and purification system, the cloned gene is inserted into a PMALTM vector downstream from the MALETM gene, which encodes maltose-binding protein (MBP). This results in the expression of an MBP-fusion protein. The technique uses the strong P_{tac} promotor and the translation initiation signals of MBP to express large amounts of the fusion protein. The PMAL-C2TM series of vectors have an exact deletion of the MALETM signal sequence, resulting in cytoplasmic expression of the fusion protein. The PMAL-P2TM series of vectors contain the normal MALETM signal sequence, which directs the fusion protein through the cytoplasmic membrane, resulting in periplasmic expression. The preferred expression system is the PMAL-C2TM vector (New England Biolabs, Beverly, MA). For insertion into this system the PCR product is digested with SspI and XbaI, purified by agarose gel electrophoresis, and cloned in a commercially available plasmid vector, PMAL-P2TM or PMAL-C2TM (New England Biolabs, Beverly, MA) which had been digested with XmnI and XbaI. This vector allows for fusion of the fifth codon of the flaa gene to an *Escherichia coli* gene encoding maltose binding protein (MBP). The MBP-FlaA fusion is transcriptionally regulated by a P_{tac} promotor and is induced by growth in isopropylthiogalactoside (IPTG). Several transformants of *E. coli* DH5-alpha, containing plasmids with the appropriate size insert, were sequenced with the MALETM primer (New England Biolabs). The MALETM primer is used for sequencing downstream from the male gene across the polylinker. One plasmid with the expected fusion-protein in the correct reading

B⁴ frame to [Male]MALETM, termed pEB11-2, was purified.

Please amend the specification at page 9, line 11:

B⁵ Expression of recombinant flaA gene in PMAL-P2/C2TM plasmid.